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Multiple shoot induction of economically significant plant *Eclipta alba* L.

KEYWORDS: Eclipta alba, Micropropagation, Plant tissue culture, Multiple shoot induction

Pushplata Dodake

Dept. of Botany and Biotechnology, Sadhu Vaswani College, Bhopal.

Malika Pal

Principal SAM Girls College Bhopal

ABSTRACT Eclipta alba is well known herbal plant for its vast range of important properties. A protocol was developed for multiple shoot induction through in-vitro culture of Eclipta alba. The Shoot tip and nodal segments of plantlets generated via seed culture were used as explants for multiple shoot induction. The explants were cultured on MS and B5 medium supplemented with different plant growth regulators (BAP, NAA, Kinetin, IAA, IBA) ranging in concentration from 0.0-4.0mg/l. About 92% regeneration and best multiple shoots were obtained by culturing the shoot tips on media supplemented with 2.omg/l BAP, although nodal segments showed almost 88% regeneration and good multiple shoots formation when media supplemented with 2.5mg/l of BAP was used. Best rooting response was observed when IAA was supplemented with half strength media. Half strength media supplemented with combinations of auxins illustrated the best root generation in Eclipta alba.

Introduction

Medicinal plants are nature's priceless gifts to human beings. The practice of traditional medicine is widespread in China, India, Japan, Pakistan, ShriLanka and Thailand. India is the largest producer of medicinal herb and can be considered as "The Botanical garden of the world". Eclipta alba is one of the endangered plant which belongs to family asteraceae. Eclipta alba, commonly known as bhringraaj is very well known herb of India especially due to its use as hair tonic. The vast range of secondary metabolites showing antimytotoxic, antimicrobial, antihepatotoxic, antioxidant, antihyperglycemic, rejuveniser and antivenom action etc. has been investigated and reported in Eclipta alba. The bioactive compounds responsible for these activities are alkaloids, flavonoids, Phenolic compouns, Tannins, Saponin etc. (Hill A F. 1952). E. prostrata has never been reported as a serious weed but it is troublesome in several crops (Holm et al., 1977).

In nature, Eclipta alba is not very fast growing and percentage of seed germination of the plant is also very low. So it is an urgency to protect this specie from getting vanished from nature. It can be obtained by mass and rapid production of the plant vai tissue culture to meet the demand. The proposed research work was also an attempt to develop and standardize a tissue culture technique for regeneration of Eclipta alba by modification of selective physical environment and culture media specific for the production of some important secondary metabolites so that the production of commercially important compounds extracted from Eclipta alba can be enhanced.

Material and Method

The plants were generated by culturing the seeds in laboratory conditions. The seeds were washed with detergent and rinsed to remove the traces. The seeds were further treated with 70% ethanol for 2 minutes and then again rinsed with sterilised distilled water. For surface sterilisation the seeds were dipped in 2% sod. Hypochlorite solution for 5 minutes and than washed with sterilised distilled water for 3-4 times to completely remove the traces of sterilizing agent. The seeds were used to generate healthy plants in sterile conditions. The Shoot tip and nodal segments of plantlets generated via seed culture were used as explants for multiple shoot induction. The explants were cultured on MS and B5 medium supplemented with different plant growth regulators (BAP, NAA, Kinetin, IAA, IBA) ranging in concentration from 0.0-4.0mg/l. The temperature maintained for culture was 25^{0}C with optimum light conditions (16/8hrs. photoperiod). Data was recorded after regular intervals.

Results

The seeds showed dormancy and only 47.5% of seeds germinated. The MS media supplemented with 0.1, 0.2mg/l BAP was found to be best suited when a series of different concentrations of hormones were tried to germinate the Eclipta alba seeds under optimum conditions. Also 20 to 25% of seed germination was observed with B5 without growth hormone and B5 supplemented with 0.1mg/l BAP respectively.

The BAP supplemented media showed 92% multiple shoot formation with maximum frequecy of 14±0.6 shoots/explant when shoot tip was taken as explant. The nodal segment exhibited 88% multiple shoot induction when cultured in MS medium supplemented with 2.5mg/l BAP (Table 1). The cultures with kinetin didn't showed significant results. A 62-64% of explants responded with low frequency of multiple shoot formation therefore this was not found the effective composition for further culture. Although when kinetin was supplemented with a fixed concentration of BAP in MS medium, the percentage response rised upto 80% and the frequency also increased up to 7-8shoots/explant. A very good regeneration rate was observed when media was supplemented with combinations of hormones. A combination of BAP and NAA (2.2 +3.5mg/l respectively) was seen to exhibit 95% shoots germination with a frequency of 9multiple shoots/explant, a combination of BA + Kin. + NAA (2.2 + 1.0 + 3.0 respectively) showed 95% germination response with a frequency of 12shoots/explant in the shoot tip cultures. The aformentioned two combinations showed 82% and 86 % shoot formation with a frequency of 9±0.6 shoots/explant and 10±0.3 shoots/explant respectively. Both the explants responded best in a medium supplemented with BAP in the same medium but highest number of microshoots were observed to be induced in shoot tips. The results were in accordance with previous studies (Gawde and Paratkar, 2004; Dhaka and Kothari, 2005; Baskaran and Jayabalan, 2005; Hussain and Anis, 2006; Han et al. 2007; Hassan et al. 2008) who reported maximum shoot proliferation could be obtained in Eclipta alba when the explants are cultured on MS medium supplemented with BAP and NAA. In

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the present study, best rooting response was observed when IAA (3.5mg/l) was supplemented with half strength MS media. Half strength media supplemented with combinations of auxins IBA + NAA, IBA + NAA, IBA + IAA with the concentrations 1.0mg/l +1.0mg/l, 1.0mg/l + 0.5mg/l and 0.5mg/l + 0.5mg/l respectively illustrated the best root generation in Eclipta alba exhibiting the root formation with 90%, 82% and 70% respectively. Therefore the Half strength medium supplemented with IAA alone or supplemented with a combination of IBA + NAA showed the best results for root formation. Raghwendra et al., 2014 reported that in vitro raised multiple shoots were transferred individually to half strength B5 medium 1.0 IBA mg/L and 0.5IBA mg/L for better root formation. Half strength B5 medium 1.0 mg/ L IBA were found to be more effective than other concentrations. Baskaran et al., (2004) achieved multiple roots (94.3 %) on full strength MS medium containing 9.8mg/L IBA (Table 2).

Conclusion

From the current study, it was concluded that MS medium supplemented with BAP and NAA serves as a best media for multiple shoot induction in Eclipta alba. The study helped to develop a standard protocol for multiple shoot induction with maximum efficiency so that mass production of Eclipta alba can be achieved within shorter span of time for commercial production.

Table1: Effect of growth regulators in MS medium on morphogenic response of *Eclipta alba* shoot tips and nodal segments

Growth Regulator	Concentration (mg/l)	Shoot Tips		Nodal Segments	
		Shoots Formation (%)	**Mean no. of shoots ± SE**	**shoot formation (%)**	**Mean no. of shoots ± SE**
BAP	0.5	39	4 ± 0.1	44	6 ± 0.1
	1.0	62	6 ± 0.8	69	7 ± 0.3
	1.5	86	10 ± 0.2	81	9 ± 0.1
	2.0	**92**	**14** ± 0.6	80	9 ± 0.7
	2.5	90	12 ± 0.1	**88**	**10** ± 0.1
Kinetin	1.0	56	4 ± 1.4	50	4 ± 1.2
	1.5	48	3 ± 0.5	41	3 ± 1.4
	2.0	62	6 ± 0.1	57	5 ± 1.2
	2.5	**64**	**6** ± 0.2	**62**	**7** ± 0.7
	3.0	60	5 ± 0.9	56	4 ± 0.4
BA + Kin	2.2 +1.0	50	2 ± 0.1	54	3 ± 0.4
	2.2 + 1.5	65	3 ± 0.3	62	4 ± 0.7
	2.2 + 2.0	70	**5** ± 1.2	62	4 ± 0.1
	2.2 + 2.5	75	4 ± 1.4	70	6 ± 1.2
	2.2 + 3.0	**80**	**8** ± 0.1	**78**	**7** ± 0.1
BA + NAA	2.2 +1.5	45	2 ± 0.8	62	4 ± 0.3
	2.2 + 2.0	55	3 ± 0.1	65	5 ± 0.3
	2.2 + 2.5	78	4 ± 0.1	65	5 ± 0.9
	2.2 + 3.0	80	4 ± 0.4	77	7 ± 1.2
	2.2 + 3.5	**95**	**9** ± 0.1	**82**	**9** ± 0.6
BA + Kin + NAA	2.2 +1.0 + 1.4	55	3 ± 0.9	64	4 ± 1.2
	2.2 +1.0 + 1.5	60	6 ± 1.0	70	5 ± 0.7
	2.2 +1.0 + 2.0	77	9 ± 0.1	72	6 ± 0.7
	2.2 +1.0 + 2.5	88	7 ± 0.8	76	8 ± 1.0
	2.2 +1.0 + 3.0	95	**12** ± 0.1	**86**	**10** ± 0.3

Table 2: Effect of different auxins on root induction in regenerated shoots of *Eclipta alba* on full and half strength MS medium

Media	Growth regulator	Amount Used (µM)	%age shoots showing root regenerat	Root Length (cm) ± SE	Days Required
Full strength	IAA	2.0	40	1.0 ± 0.1	12 ± 0.1
		2.5	55	1.5 ± 0.4	16 ± 1.4
		3.0	60	3.0 ± 0.1	14± 0.1
		3.5	62	3.0 ± 0.1	12 ± 0.1
		4.0	**65**	**4.0** ± 0.3	10 ± 0.1
Full Strength MS	NAA	2.5	65	4.0 ± 0.8	3 ± 0.1
		3.0	70	4.0 ± 0.2	23 ± 0.3
		3.5	75	4.5 ± 0.1	18 ± 1.4
		4.0	**80**	**5.2** ± 1.0	10 ± 0.8
		4.5	85	5.1 ± 0.5	14 ± 0.1
	IBA	**0.5**	**80**	4.5 ± 0.5	9 ± 1.0
		0.75	71	2.4 ± 0.1	9 ± 1.0
		1.0	60	2.8 ± 0.7	12 ± 0.5
Half Strength MS	IAA	2.0	40	1.5 ± 1.2	15 ±0.3
		2.5	45	2.5 ± 0.1	12 ±0.5
		3.0	50	3.1 ± 0.3	18 ±0.3
		3.5	**90**	3.4 ± 0.1	8 ±0.3
		4.0	65	3.1 ± 0.8	26 ± 0.1
Half Strength	NAA	2.5	55	3.2 ± 1.6	20 ± 0.1
		3.0	60	3.2 ± 1.2	24 ± 0.8
		3.5	65	4.1 ± 0.9	24 ± 0.5
		4.0	**70**	**4.3** ± 0.1	15 ± 1.0
		4.5	75	4.1 ± 0.7	21 ± 0.1
	IBA +NAA	**1.0 + 1.0**	**90**	**4.8** ± 1.2	14 ± 0.8
	IBA + NAA	**1.0 + 0.5**	**82**	**5.1** ± 0.8	13 ± 1.4
	IBA + IAA	**0.5 + 0.5**	**70**	**4.9** ± 1.5	21 ± 0.8

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